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(54) Title: MONOCLONAL ANTIBODIES SPECIFIC FOR PROSTATE SPECIFIC ANTIGEN AND METHODS OF DETECTING PROSTATE SPECIFIC ANTIGEN

(57) Abstract

The present invention is directed to monoclonal antibodies useful in detecting prostate specific antigen. Several monoclonal antibodies bind free and bound prostate specific antigen and one of the monoclonal antibodies binds free prostate specific antigen. In addition, methods of detecting cancer, including prostate cancer, are embodied by the present invention.

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MONOCLONAL ANTIBODIES SPECIFIC FOR PROSTATE SPECIFIC ANTIGEN AND METHODS OF DETECTING PROSTATE SPECIFIC ANTIGEN

BACKGROUND OF THE INVENTION

Prostate specific antigen (PSA) is a serine protease of 237 amino acids, with one N-linked and three O-linked carbohydrate side chains. Prostate specific antigen is a 33-34 kD protein produced by prostatic epithelial cells. Surrounding the neck of the bladder and the urethra in men, the prostate is part muscular and part glandular, with ducts opening into the prostatic portion of the urethra. The gland secretes a thin, opalescent, slightly alkaline fluid that forms part of the seminal fluid. PSA is a major protein component of this fluid.

PSA is a member of the kallikrein family along with pancreatic/renal kallikrein, and glandular kallikrein of the prostate (hGK-1 or hK-2). PSA and hGK-1 have 78% protein sequence homology, and their genes are found in tandem, 12 kb apart on chromosome 19. PSA functions as a chymotrypsin-like protease, and the identified targets are seminogelin I and II, and fibronectin. Cleavage of seminogelin allows liquefaction of seminal coagulum.

PSA is a marker to detect and monitor prostate cancer and benign prostatic hyperplasia. It is also found in small quantities (0-4 ng/ml) in serum, but if the gland is inflamed or cancerous, the serum levels increase. PSA is found in serum in both a free form and bound (or complexed) with alpha-1-antichymotrypsin (ACT), an endogenous serine protease inhibitor. The majority of serum PSA is found complexed to serine protease inhibitors alpha-1-antichymotrypsin (ACT; resulting in a Mr of 90 kD), or alpha-

2-macroglobulin (AMG; resulting in a Mr of 750 kD). PSA bound to AMG is not immunogenic probably because the AMG engulfs PSA.

PSA was cloned from cDNA libraries by Lundwall and Lilja (1987) and a longer PSA sequence was cloned by Henttu and Vihko (1989). The disclosures of each of the references cited herein are incorporated herein by reference in their entirety. Total genomic DNA was searched and the PSA gene cloned by Lundwall (1989). hGK-1 was cloned from a genomic library by Schedlich et al. (1987).

Benign prostatic hyperplasia (BPH) is common to nearly all men over 60, and occurs in younger men as well. Because of the location of the gland, symptoms include the need for frequent nocturnal urination. Prostatic cancer (PCa) is the most common non-skin cancer in men, the fourth most common cause of death in the United States. In 1994, approximately 38,000 men died of PCa. The combination of PSA serum level testing and digital rectal exam (DRE) is sufficient for diagnosis in many of the cases. The treatment of PCa is radical prostatectomy in which the prostate gland, seminal vesicles, and narrow cuff of bladder neck are removed. The chance of impotence has decreased from 100% to 30%-50% in the last five years as surgery was developed to try to save the capsular and periprostatic nerves. There is also a chance of incontinence.

There are 18 million men in the united States 50-70 years old that should be screened for PSA levels annually. Of these only 8-10 million men comply. Within this population, 10% (approximately 1 million men) will have PSA values between 4-10 ng/ml, a range where BPH and PCa are indistinguishable by total PSA testing. Currently this group is biopsied, but the inconvenience and expense is encouraging the search for better and different PSA testing. PSA levels should also be followed after radical prostatectomy to predict persistent disease.

Separation of BPH and PCa patients with PSA values between 4 and 10 ng/ml have been reported by comparing the serum PSA circulating free (Mr 30 kD) and complexed with ACT (Mr 90 kD), and the combination of free and complexed or "Total PSA". The ratio of free/total yields PCa patients with a lower mean than BPH patients. This ratio approach is achieved with antibodies to the common site of free and complexed PSA (anti-total antibody), and an antibody to the ACT binding site of PSA which is obscured with ACT binding (anti-free antibody).

Since PSA and hGK-1 are so similar, it is now being considered that antibodies for total PSA are also identifying hGK-1. To the knowledge of the inventor, no correlation of hGK-1 with disease has been discovered, and no assay has been formulated. Recently, PSA immunoreactivity was found in breast cancer, Yu, H., et al. (1995). Nonetheless, in spite of the different forms and species of PSA and PSA-like molecules in the circulation, many if not most anti-PSA antibodies used in commercial PSA assays appear to react equally with all forms. Development of reagents to individually recognize these specific forms to define the fine specificity of different antibodies to PSA and PSA-like molecules.

There remains a need for a PSA assay which will aid in the evaluation of PSA free/total ratio, define the current anti-PSA antibodies' epitopes and any subsequently isolated antibodies, including anti-free PSA, anti-bound PSA, and anti-total PSA antibodies. The present invention was developed using a series of 15-mers overlapping by 12 amino acids to define areas of reactivity for antibodies recognizing predominantly conformational epitopes. Using synthetic peptides in an ELISA format, epitopes were identified that were recognized by both monoclonal antibodies and polyclonal antisera to PSA. Using homology modeling, a structure of PSA was constructed which revealed a loop in the three dimensional structure of free PSA. The epitopes identified were mapped to the PSA model.

SUMMARY OF THE INVENTION

The present invention is directed to monoclonal antibodies that bind to determinants found in free and bound prostate specific antigen, hereinafter denoted anti-PSA antibodies. A monoclonal antibody which binds free prostate specific antigen at a determinant comprising amino acids 82-87 of free prostate specific antigen is provided in the present invention. A hybridoma producing the monoclonal antibody that binds amino acids 82-87 of free prostate specific antigen and the monoclonal antibody produced therefrom are also embodiments of the present invention.

The present invention is directed to monoclonal antibodies that bind to amino acids 139-144 or a fragment thereof of prostate specific antigen, a hybridoma producing a monoclonal antibody which binds amino acids 139-144 or a fragment thereof of prostate specific antigen, and a monoclonal antibody produced therefrom.

Also embodied by the present invention are monoclonal antibodies that bind to amino acids 55-60 or a fragment thereof of prostate specific antigen, a hybridoma producing a monoclonal antibody which binds amino acids 55-60 or a fragment thereof of prostate specific antigen, and a monoclonal antibody produced therefrom.

The monoclonal anti-PSA antibodies of this invention are useful in a number of diagnostic and therapeutic applications, including methods for the detection of cancer in mammals. The present invention also provides methods that permit the discovery of prostate specific antigen in a sample. The prostate specific antigen may be free, bound, or total.

In addition, kits for the detection of free, bound, and/or total prostate specific antigen comprising an immunoabsorbant, labeled monoclonal antibody specific for free, bound, and/or total prostate specific antigen, and a control are also provided in the present invention.

Another embodiment of the present invention is a method of screening for monoclonal antibodies specific for amino acids 82-87 or a fragment thereof of free prostate specific antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 displays the three dimensional backbone structure of serine proteases thrombin, α-thrombin, trypsin, epsilon-thrombin, elastase, trypsinogen, tonin, chymotrypsinogen, kallikrenin, and rat mast cell protease. The catalytic triad, represented by three yellow circles, is found in the center of the structure.

Figures 2A and 2B display the epitope map of anti-PSA antibodies of monoclonal antibodies 10, 11, 15.2, 16, 22.2, 156, 225, and 365. A series of 75 15-mer peptide sequences covered the entire sequence of PSA.

Figure 3 displays an alignment of the protein sequences of PSA, SEQ ID NO: 18, and hK-2, SEQ ID NO: 94. Epitopes in PSA recognized by anti-PS monoclonal antibodies and polyclonal antisera are underlined.

Figure 4 displays the sequences of PSA peptides synthesized as 15-mers.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a monoclonal antibody that binds to

amino acids 82-87, 169-171, and 223-228 of free prostate specific antigen (PSA). The amino acids 82-87 are in a loop region evident in the tertiary structure of free prostate specific antigen, see Figure 1. While not intending to be bound by any particular theory of operation, it is believed that amino acids 82-87 of the loop region of prostate specific antigen are conformationally available when PSA is in the free form, that is, PSA not bound or complexed to α-1-antichymotrypsin (ACT). While free and bound PSA bind amino acids 169-171 and 223-228, amino acids 82-87 distinguish free PSA from bound PSA. Amino acids 82-87 are thus surface exposed in free PSA. The monoclonal antibody found to bind to amino acids 82-87 is designated herein as monoclonal antibody 365. The antigenic/immunogenic peptide also identified herein as the epitope, target antigen, or determinant of PSA to which monoclonal antibody 365 binds comprises the amino acid sequence LKNRFL (SEQ ID NO. 1), amino acids 82-87. The epitope may be contained within the six amino acid sequence of SEQ ID NO: 1. Thus, a fragment of the PSA sequence amino acids 82-87 is also an embodiment of the present invention. For purposes of the present invention, fragment refers to part of the amino acid sequence of SEQ ID NO: 1 substantially similar thereto, to which PSA specifically or immunologically binds, which fragment thus has PSA specific activity. The present invention also includes monoclonal antibodies having characteristics of monoclonal antibody 365. Peptide 15-mers having the sequence of SEQ ID NO: 1 or a fragment thereof are peptides 24-29 set forth in Figure 4.

Monoclonal antibodies that bind the amino acid sequence 55-60, SLFHPE (SEQ ID NO: 2), or a fragment thereof of prostate specific antigen are also embodied by the present invention. The monoclonal antibodies are monoclonal antibodies 15.2, 156, and 225, or monoclonal antibodies having the characteristics of any of these foregoing monoclonal antibodies. Peptide 15-mers having the sequence of SEQ ID NO: 2 are peptides 16-19 of Figure 4.

Monoclonal antibodies 10, 11, 16, 22.2 or monoclonal antibodies having the characteristics of any of these foregoing monoclonal antibodies which bind amino acids 139-144, EELFLTP (SEQ ID NO: 3), or a fragment thereof of prostate specific antigen are within the scope of the present invention. Peptides 44-47 of Figure 4 include the sequence of SEQ ID NO: 3.

Hybridomas capable of producing any of the monoclonal antibodies identified herein are also embodied by the present invention.

The term "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. Of these, IgM and IgG are particularly preferred. The antibodies may be monoclonal or polyclonal and may be of any species of origin, including (for example) mouse, rat, rabbit, horse, or human, or may be chimeric antibodies. See, for example., Walker et al. (1989). The antibodies may be recombinant monoclonal antibodies produced according to the methods disclosed in Reading, U.S. Patent No. 4,474,893, or Cabilly et al., U.S. Patent No. 4,816,567. The antibodies may also be chemically constructed by specific antibodies made according to the method disclosed in Segel et al., U.S. Patent No. 4,676,980.

Antibodies other than the antibodies set forth above which bind the antigen bound by the above-identified antibodies may be obtained in accordance with known techniques. For example, cells identified as carrying the antigen having amino acids 82-87 bound by the 365 antibody can be washed with an aqueous solution containing a detergent to remove the antigen therefrom, the various fractions in the solution separated by chromatography (e.g., high performance liquid chromatography), and the fraction containing the antigen identified by its ability to bind the antibody. In the alternative, the antibody may be immobilized on a solid support to provide an affinity chromatography column, a sample, such as and not limited to a serum sample, from an appropriate patient (e.g., a patient diagnosed as carrying free prostate specific antigen) passed through the column, antigen bound to the antibody eluted from the column, and the antigen used to produce an antibody. Thus, disclosed herein is a purified antigen produced by the foregoing processes. Such an antigen is recognized by monoclonal antibody. The antigen may be provided in an aqueous carrier, or in lyophilized form. The antigen may be used to immunize animals to produce polyclonal antibodies, or may be used as an assay standard. The antibodies to an antigen having amino acids 55-60 or an antigen having amino acids 139-144 of prostate specific antigen may be prepared with known techniques similar to those set forth above.

Antibodies which bind to the epitope (i.e., the specific binding site) bound

by any of the above-identified antibodies can be identified in accordance with known techniques, such as their ability to compete with labelled antibody in a competitive binding assay.

Antibody fragments included within the scope of the present invention include, for example, Fab, F(ab')2, and Fv fragments, and the corresponding fragments obtained from antibodies other than IgG. Such fragments can be produced by known techniques.

Polyclonal antibodies used to carry out the present invention may be produced by immunizing a suitable animal (e.g., rabbit, goat, etc.) with an antigen to which a monoclonal antibody binds, collecting immune serum from the animal, and separating the polyclonal antibodies from the immune serum, in accordance with known procedures.

The monoclonal anti-PSA antibodies are produced by antibody-producing cell lines. The anti-PSA antibody-producing cell lines may be hybridoma cell lines commonly known as hybridomas, produced according to the technique of Kohler and Milstein (1975). The hybrid cells are formed from the fusion of an anti-PSA antibody-producing cell and an immortalizing cell line, that is, a cell line which imparts long term tissue culture stability on the hybrid cell. In the formation of the hybrid cell lines, the first fusion partner, the anti-PSA antibody-producing cell, may be a spleen cell of an animal immunized against PSA. Alternatively, the anti-PSA antibody-producing cell may be an anti-PSA generating B lymphocyte obtained from the spleen, peripheral blood, lymph nodes or other tissue. The second fusion partner, the immortal cell, may be a lymphoblastoid cell or a plasmacytoma cell such as a myeloma cell, itself an antibody-producing cell but also malignant.

Murine hybridomas which produce monoclonal anti-PSA antibodies are formed by the fusion of mouse myeloma cells and spleen cells from mice immunized against PSA. To immunize the mice, a variety of different immunization protocols may be followed. For instance mice may receive primary and boosting immunizations of PSA. The fusions are accomplished by standard procedures such as those disclosed by Kohler and Milstein (1975) and Kennet (1980).

The hybridomas are then screened for production of antibody reactive with PSA in accordance with standard methods such as those disclosed by Tam (1994). Those which secrete reactive antibodies are cloned.

Human hybridomas which produce monoclonal anti-PSA antibodies are formed from the fusion of spleen cells from an individual immunized against PSA and a human lymphoblastoid cell line. Alternatively, the fusion partner for the myeloma cell may be a human peripheral blood antibody-producing lymphocyte sensitized against PSA. The fusion and screening techniques are essentially the same as those used in the production and selection of murine anti-PSA generating hybridomas.

Also mouse and human hybridomas which produce human monoclonal anti-PSA antibody may be formed from the fusion of a human antibody-producing cell and a murine plasmacytoma cell. Indeed, the mouse plasmacytoma cell may be used as a fusion partner for other mammalian antibody-producing cells to form hybridomas which produce anti-PSA antibody of the particular mammal.

Another way of forming the anti-PSA antibody-producing cell line is by transformation of antibody-producing cells. For example, an anti-PSA antibody producing B lymphocyte obtained from an animal immunized against PSA, may be infected and transformed with a virus such as the Epstein-Barr virus in the case of human B lymphocytes to give an immortal anti-PSA antibody-producing cell. See, for example, Kozbor and Roder (1983). The B lymphocyte may be alternatively transformed by a transforming gene or transforming gene product.

The monoclonal anti-PSA antibodies are produced in large quantities by injecting anti-PSA antibody-producing hybridomas into the peritoneal cavity of mice and, after an appropriate time, harvesting the ascites fluid which contains very high titer of homogenous antibody and isolating the monoclonal anti-PSA antibodies therefrom. Xenogeneic hybridomas should be injected into irradiated or athymic nude mice. Alternatively, the antibodies may be produced by culturing anti-PSA producing cells *in vitro* and isolating secreted monoclonal anti-PSA antibodies from the cell culture medium.

Monoclonal Fab fragments may be produced in *Escherichia coli* by recombinant techniques known to those skilled in the art. See, for example, Huse (1989).

The monoclonal anti-PSA antibodies of this invention have a number of important therapeutic uses. Because of their restricted specificity for PSA, the anti-PSA antibodies of this invention may be used to detect and measure free, bound, and total (free plus bound) PSA in a patient sample such as and not limited to fluid including blood, serum, urine, and a tear.

For this purpose, the antibodies may be used in conventional immunoassays such as radioimmunoassay or enzyme linked immunoadsorbent assay. For many of these assays an immunoadsorbent is formed by attaching anti-PSA antibody to a solid phase. In a competitive immunoassay for PSA, for example, a sample of the biological fluid to be assayed is contacted with the immunoadsorbent.

The mixture is incubated after which a predetermined amount of labeled PSA is added. After further incubation, the immunoadsorbent with the affixed PSA (that is, the PSA anti-PSA complex) is separated from the PSA not affixed thereto and the activity of the label in the affixed PSA fraction is measured in order to determine the amount of PSA in the sample. In such assays, the label may be a radioisotope, an enzyme, or a fluorescent compound.

The anti-PSA antibodies can be provided as reagents in kits for radioimmunoscintigraphy in mammals. Such kits would include the labeled monoclonal anti-PSA antibody or fragments of the antibodies, labeled for example with one of the gamma-emitting radioisotopes.

A method of screening for cancer in a patient comprising contacting a patient sample with a monoclonal antibody specific for amino acids 82-87 or a fragment thereof of free prostate specific antigen under conditions permitting the monoclonal antibody to specifically bind an antigen in the sample to form a first antibody-antigen complex; contacting the sample with a monoclonal antibody specific for free and bound prostate specific antigen under conditions permitting the antibody to specifically bind an antigen in the sample to form a second antibody-antigen complex; determining the amount of the first antibody-antigen complex in the sample; determining the amount of the second antibody-antigen complex in the sample; determining the ratio of the first antibody-antigen complex to the second antibody-antigen complex.

The method of detecting cancer includes the detection of prostate cancer and breast cancer. In addition the method may be used to determine the presence of benign prostatic hyperplasia (BPH) in a patient. Monoclonal antibodies useful for the detection of free PSA include monoclonal antibodies binding to SEQ ID NO: 1, such as a monoclonal antibody having the characteristics of monoclonal antibody 365. Monoclonal antibodies useful for the detection of total PSA include monoclonal antibodies binding to SEQ ID NOS: 2 and 3, such as a monoclonal antibody having the characteristics of a

monoclonal antibody selected from the group consisting of monoclonal antibodies 10, 11, 15.2, 16, 22.2, 156, and 225.

The ratio of free to total PSA of a patient of about 4 to about 10 ng/ml currently require biopsy to determine if the patient has prostate cancer of BPH. Accordingly, the monoclonal antibodies of the present invention, particularly 365 which is specific for free PSA, provide a more accurate diagnosis of prostate cancer or BPH than currently available techniques.

The first and second monoclonal antibodies useful in the method of detecting cancer may be labeled such that the label of the first monoclonal antibody is distinguishable from the label for the second.

Diagnostic kits for performance of the assays described above include monoclonal anti-PSA antibody or labeled anti-PSA antibody or mixtures of labeled or unlabeled antibody, and a PSA control.

The assays described herein provide physicians with a quick and reliable method of ruling out prostate cancer or benign prostatic hyperplasia in patients. Current techniques for determining a clinical diagnosis of prostate cancer or benign prostatic hyperplasia are time consuming. The monoclonal antibodies, kits, and methods of the present invention would avoid delaying treatment of a cancerous disease.

Assays of the present invention quickly rule out prostate cancer and avoid subjecting patients to painful biopsy and the attendant risk of complications, without any significant delay in treatment of those patients who have prostate cancer.

The following examples are illustrative but are not meant to be limiting of the invention.

EXAMPLES

Preparation of Mouse Immune Splenocytes

Five 12-13 week old female Balb/c mice obtained from Charles River Laboratories were immunized according to the method of Cianfriglia (1984). Mice were injected IP with 50 μg PSA (Scripps) emulsified with an equal volume of Complete Freunds adjuvant (Gibco) in a final volume of 250 μL on day 1 and day 8. All mice were boosted IP on day 13 with 50 μg PSA (Scripps) diluted in 250 μL in Dulbecco's phosphate buffered saline (PBS). On day 14, mice received 10 μg PSA in 250 μL PBS administered

IP and 10 μg PSA in 100 μL PBS given IV. For fusion #1, mouse #3 was sacrificed by cervical dislocation on day 16 and the spleen was removed aseptically and immersed into 10 mL of cold PBS containing 100U penicillin, 0.1 mg streptomycin, and 0.25 μg amphotericin B (PSA) (Sigma).

The splenic B cells were harvested by sterilely perfusing the spleen with PSA-PBS. The cells were washed once in cold PSA-PBS, counted and diluted to $2x10^7$ cells/mL. The cells were then layered into Lympholyte-M (Cedar Lane Laboratories Limited) and centrifuged at 500 g for 20 minutes at RT to separate out the lymphocytes. The lymphocyte layer was carefully removed and the cells were washed in PSA-PBS.

For fusion #2, the remaining mice were reimmunized IP on day 49 with 100 μg PSA emulsified in an equal volume of Incomplete Freunds adjuvant (Gibco) in a final volume of 250 μL. On day 126, all mice were again boosted IP with 50 μg PSA (Scripps) diluted in 250 μL PBS. Two weeks later, the mice were bled by retro-orbital stick without anti-coagulant. The blood was allowed to clot at room temperature and the serum collected. A solid phase EIA-HRP was run to titer the mouse immune serum for anti-PSA activity. On day 150, 2 mice (#1 and #2) were boosted IV with 25 μg PSA in 250 μL PBS. Three days after injection, mouse #2 with a titer $>1:200,000$ was sacrificed by cervical dislocation and the spleen was prepared as described for fusion #1.

Murine Myeloma Fusion Partner

A cell bank of the non-secreting Balb/c mouse myeloma fusion partner, FO (Fazekas de St. Groth (1980)) was purchased from ATCC (#CRL-1646). One frozen vial of FOs was thawed and resuspended in αMEM medium (JRH Biosciences) supplemented with 10% FBS (lot #20219-02, Cell Culture Labs), 1 mM sodium pyruvate, 0.1 mM NEAA, 2 mM L-glutamine (all from JRH Biosciences). The cells were expanded, cryopreserved in 95% (v/v) FBS and 5% (v/v) DMSO (Sigma) and kept under nitrogen conditions in CBS. The cell bank was sterile and free of mycoplasma (Tektagen). Several days prior to either fusion, the FO cells were thawed and cultured in the media described above. Cells were kept in log phase. They were washed in PBS, counted and viabilities determined to be at least 95% via trypan blue dye exclusion prior to fusions.

Fusion

Both fusions were carried out at a 2:1 ratio of murine myeloma cells to viable spleen cells according to the method of Fazekas de St. Groth. Briefly, approximately $2x10^8$ spleen cells and $4x10^8$ myeloma cells were pelleted together. The pellet was gently resuspended over 1 minute in 2 mL of 50% PEG solution (5 g PEG 3,000 (Sigma), 5 mL dH_2O (Baxter), 500 μL DMSO (Sigma) at 37°C. The PEG/cell mixture was then immersed in a 37°C water bath for 1.5 to 2 minutes with gentle agitation. The fusion was stopped by slowly adding incremented volumes of PBS (37°C) over 2 minutes. The fused cells were allowed to rest for 5 minutes at RT and pelleted. The cells were washed once in HAT medium (αMEM, 20% FBS, 1 mM sodium pyruvate, 0.1 mM NEAA, 2 mM L-glutamine, 25 μg/mL gentamicin (Sigma)), and HAT (100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine, (Sigma)) and then plated at 100 μL/well in 25 96-well flat bottom plates (Corning) which contained 50 μL/well PECs as feeder cells. PECs were prepared by lavaging the peritoneum of naive Balb/c mice with 10 mL of ice cold PSA-PBS. PECs were washed twice and plated in HAT media 2 days prior to fusion. The plates were then placed in a humidified 37°C incubator containing 5% CO_2 and 95% air for 7-10 days.

Primary Screening

Both fusion #1 (referred to as fP hybridomas) and fusion #2 (referred to as PSA hybridomas) were assayed for IgG reactivity against free or bound PSA on a solid phase EIA.

Polyclonal sheep anti-human PSA IgG (The Binding Site) was coated into wells of 96-well flat bottom EIA plates (Corning) at 10 μg/ml in 10 mM carbonate-bicarbonate buffer, pH 9.6, at 100 μl/well for six hours, at room temperature. After washing with 0.05% tween 20 in H_20), plates were blocked with 1% BSA (Intergen) in PBS, at 250 μl/well overnight at 4°C. The blocker was removed and the dilution of either purified PSA (Scripps) or complexed PSA-ACT (present in prostate cancer patient serum) in Incubation buffer (Boehringer-Mannheim) were added to the plates at 2 ng/ml and 35.4 ng/ml, respectively and incubated for two hours, followed by washing as above. The hybridoma supernatants or control antibody dilutions were added at 50 μl/well, and allowed to react for 3 hours at room temperature. The plates were washed as above and

50 μl/well of HRP-conjugated sheep anti-mouse IgG (ICN) at 1:500 dilution in 1% BSA-PBS was added and incubated for one hour at room temperature. After washing, the reaction was visualized by adding 100 μl of OPD (Sigma) solution in 25 mM citric acid, 50 mM sodium phosphate, 10 mM 2-chloracetamide and 0.01% H_2O_2 and incubated for 30 minutes in the dark. The reaction was terminated by adding 50 μl of 4N H_2SO_4 and the OD was determined at 490-650 nm using a Kinetic Microplate Reader (Molecular Devices).

The antibodies from fusion #1 and fusion #2 that were reactive equally to both free PSA and complexed PSA (cancer serum) were considered as having anti-total PSA reactivity and those preferentially reactive to only free PSA were considered as having anti-free PSA reactivity. The monoclonal antibodies 156, 15.2, and 22.2 were determined as anti-total PSA clones, the monoclonal antibodies 365, 225, 10, 11, and 16 as anti-free PSA clones.

All wells were microscopically examined after 7-10 days in culture for viable hybridoma colonies. Supernatants were removed and tested as previously mentioned. Specific fP or PSA hybridoma cell lines were chosen, expanded in tissue culture or ascites and subcloned by limiting dilution. Stable cell lines with appropriate specificities were identified and referred to as fP165 (from fusion #1) and PSA3 and PSA15 (from fusion #2). They were cryopreserved in freezing medium (95% FBS, 5% DMSO) and stored in liquid nitrogen.

General Materials

Streptavidin coated microtiter plates were obtained from Labsystems. HRP-conjugated goat anti-mouse IgG was purchased from Pierce. O-phenylene diamine was purchased from Sigma.

Peptide Synthesis

Peptides were synthesized on an ACT396 using FMOC chemistry on Rink resin and the standard double coupling DIC/HOBt protocol. 15-mers overlapping by 12 amino acids were synthesized covering the entire length of PSA. The sequence of PSA is that disclosed by Lundwall, 1989. All cystines were replace with α-amino-L-butyric acid. After completion of the 15th amino acid coupling, FMOC-L-Lys (biotin) was coupled to

the peptide resin. Deprotection and subsequent acetylation gave the fully protected peptide amide resins. Peptides were simultaneously cleaved from the resin and deprotected using 1 ml of a mixture of 22.5 g phenol, 15 g dithiothreitol, 15 ml thianisole, 15 ml water and 300 ml trifluoroacetic acid for 2 hours at ambient temperature. The resin was removed by filtration and the resulting solution added to 15 ml methyl-t-butylether. The precipitated peptide was isolated by centrifugation,, washed three times by resuspension in methyl-t-butylether with vigorous vortexing followed by centrifugation and dried under reduced pressure at ambient temperature. The peptides were used without further purification.

ELISA Assay

Streptavidin coated microtiter plate (Labsystems) was used as a solid phase. Fifty µl of peptide (concentration 3.5 µM in % BSA in PBS) was added to the wells and incubated at room temperature for three hours. After this, and every consecutive incubation step, the plates ere washed three times with deionized water containing 0.05% Tween-20. Fifty µl culture supernatant containing the test antibodies in 1 to 10 µg/ml concentrations was added to the wells and incubated overnight (16 hours) at 4°C. The reaction was detected by consecutive incubations with an HRP-conjugated goat anti-mouse IgG (H+L specific) reagent Pierce, 1:10,000 dilution, 50 µl/well, 90 minutes at room temperature) and 100 µl substrate-chromogen reagent (1 mg/ml o-phenylene diamine (Sigma) in citrate buffer, 0.4 µg/ml hydrogen peroxide, 30 minutes). The reaction was terminated with 50 µl 4 NH_2SO_4 and the absorbance read at 490 µm-650 µm wavelength using a microtiter plate reader (Molecular Devices). Buffer without peptide was used as a negative antigen control and an unrelated mouse antibody was used as a negative control for the test antibodies. A purified PSA preparation (10 ng/ml) captured by an affinity purified goat anti-PSA antibody) passively coated at 2 µg/ml concentration) served as a positive control. The reaction of an antibody to a peptide sequence was considered significant only if the absorbance exceeded the respective background level by at least three fold and at least two consecutive peptide sequences reacted.

Molecular Model of PSA

Modeling was done using Sybyl 6.2 (Tripos) on a Silocon Graphics Crimson R4400-150 workstation. The crystal structure of the serine protease tonin

(PDB1TON.ENT) was used as a template. The MegAlign module from DNAx was used to align the sequences of tonin and PSA. Sequence numbering of PSA is used throughout. Amino acids were individually mutated from the tonin to the PSA sequence and inspected visually for potentially unfavorable side chain steric interactions. These were adjusted using the conformation side chain relaxation protocol. Two amino acid sequences of PSA lack homologous regions in tonin (21-22 and 83-88). Amino acids 21 and 22 were inserted from the corresponding sequence of kallikrein. The kallikrein (13-29) sequence PWQVAIYHYSSFQCGGV, SEQ ID NO: 4, was extracted. A least squares fit was done on the alpha carbons of PWQV, SEQ ID NO: 5, and CGGV, SEQ ID NO: 6, of kallikrein and the corresponding sequences of the PSA construct, WQVL, SEQ ID NO: 7, and CGGV, SEQ ID NO: 8, (RMS 0.224 A). The PSA sequence WQ...GG was excised and replaced with the WQ...GG kallikrein loop. Amino acids were mutated and side chains adjusted were necessary. No good homology match for the seven amino acids sequence 83-88 was found in the crystal structure of any serine protease. A loop search of the Brookhaven database was done using Pro^{75} and Ser^{93} as the anchoring residues. 25 loop structures were identified. Each of these loops was inserted into the PSA construct, side chains relaxed and charges added to the entire molecule. The inserted loop was subjected to 100 cycles of minimization using the steepest descent method with a dielectric constant of 10. This was followed by 100 fs of random velocity dynamics (300 K, 5 atm) on the inserted loop and an additional 100 cycles of minimization. The entire molecule was then subjected to 100 cycles of minimization. The 25 structures coalesced into four families of similar energy, one concatenated structure and three with large loops having no interactions with the rest of the structure. Two of the families of structures of similar energy have the 83-88 sequence buried within the molecule. Since this sequence is an epitope recognized by some of the anti-PSA antibodies, these structures are inconsistent with the mapping data. The remaining two families of structures have the 83-88 loop in distinctly different conformations. In both of these structures the catalytic triad of His^{57}, Asp^{102}, and Ser^{195} is well conserved and accessible for substrate interactions. In neither of these structures are the loops identical with the one in the theoretical structure PDB1PSA.ENT in the Brookhaven crystallographic database. Examination of the crystal structures of the various serine proteinases in the crystallographic data base shows that, while the overall backbone structures of serine proteinases are well conserved, this loop is generally conformationally

unique. The inability to use homology modeling to construct this loop is consistent with the results of others using the InsightII modeling package from Biosym Technologies, Inc.

Seventy five peptide amides, each containing 15 amino acids from the PSA sequence and an additional acetyl-L-lysine (biotin) at the N-terminus were synthesized using automated solid phase synthesis and FMOC chemistry. All cystines were replaced with α-amino-L-butyric acid to avoid intramolecular disulfide formation or dimerization. Random analysis of peptides by mass spectrometry showed that the correct biotinylated peptides were present. HPLC showed varying degrees of purity. The most common impurity as determined by mass spectrometry was deletion of the acetyl-lysine (biotin) from the N-terminus, giving an impurity that would not be captured by streptavidin. Each sequential peptide overlapped 12 amino acids of its predecessor (frame shift of 3 amino acids). This high degree of overlap means that potential hepta and hexa and penta and tetrapeptide epitopes will be present in up to four adjacent peptides and tripeptide epitope in five adjacent peptides; however, epitopes could be present in as few as two adjacent peptides. Table 1 gives the sequences of the peptides synthesized, omitting the N-terminal acetyl-lysine (biotin) and the C-terminal amide.

A theoretical model of PSA was constructed using homology modeling. The majority of the frameworks of the serine proteases that have been crystallized are structurally similar as shown in Figure 1 as is the position of the catalytic triad of AspHisSer. Tonin was chosen based on the highest sequence homology with PSA. Based on the procedures described above, two possible structures for PSA were generated. It is not possible to distinguish whether either of these is more probable based on structural differences since this region is highly variable in many of the serine proteases. The usefulness of this model is in the identification of surface exposed residues and correlating them with experimentally determined epitopes. Surface exposed amino acids were identified visually.

Six biotinylated peptides were selected from different regions of PSA to determine the optimum coating concentration. Peptides were dissolved in 1% BSA in PBS. From an initial concentration of 70 μM, 5 fold serial dilutions were made to a concentration of 4.5 nM. Titration curves were generated and it was determined that peptide concentrations of 3.5 μM were sufficient to saturate all biotin binding sites on the streptavidin plates. Incubation times between 3 hours at ambient temperature and overnight

at 4°C gave identical results. All subsequent studies were done using peptide concentrations of 3.5 μM.

The results of all monoclonals and polyclonal antisera tests are summarized in Figures 2A and 2B with the solid rectangles indicating significant antibody binding to peptides. The detailed results from mouse #2 polyclonal antisera are shown in Figures 2A and 2B and are representative of the signal strength obtained for all polyclonal antisera. Peptides 17-19, 32-33, 44-47, and 53 show significant binding to the antisera. Signal clusters 17-19, 32-33, 44-47 are consistent with the type of pattern expected for epitope mapping with closely overlapping peptides. Binding to the isolated peptide 53 is reproducible. The common sequence of peptides 17-19 is SLFHPEDTG, SEQ ID NO: 9 (55-63), a sequence that is surface exposed on the PSA model. The common sequence to peptides 32-33 is LMLLRLSEPAEL, SEQ ID NO: 10 (97-108), but only SEPAEL, SEQ ID NO: 11 (103-108) is surface exposed. The common sequence of peptides 44-47 is the surface exposed EEFLTP, SEQ ID NO: 12 (140-145). Peptide 53, NDVCAQVHPQKVTKF, SEQ ID NO: 13 (158-172), is completely surface exposed.

Low background and the characteristic cluster of peptides that bind with these antibodies, identifies a single sequential epitope. Monoclonal 15.2 recognizes the surface exposed sequence SLFHPEDTG, SEQ ID NO: 9 (55-63) defined by peptides 17-19 and monoclonal 16 recognizes LTP (143-145), the sequence common to peptides 44-48. These are similar to the epitopes defined by the mouse #2 polyclonal antisera. Although not spatially adjacent, the distance between these two epitopes is such that antibodies recognizing one epitope could be experimentally competitive with antibodies recognizing the other. In addition to the binding clusters at peptides 55-57 and 72-74, the antibody also shows reproducible binding to peptide 14. Of the sequence common to peptides 55-57, only TKF (170-172) is surface exposed and peptides 72-74 define the surface exposed sequence VHYRKW, SEQ ID NO: 14 (224-229). Although not sequentially contiguous, these two sequences are topologically adjacent and define a single conformational epitope. Although peptide 14 is totally surface exposed, its distance from the other two sequences is larger than the size of an antibody binding domain.

The anti-PSA monoclonal antibodies recognizing linear sequences bind to peptides identifying two epitopes. These fall within peptides 16-19 and 44-47. Although similar, the epitopes are not identical. For example, the epitope of monoclonal is 15.2 is

defined by peptides 17-19 while that of monoclonal 225 is defined by peptides 16-18. Although these two antibodies would be expected to compete, the epitopes are not identical on the amino acid level. The epitopes of the polyclonal antisera are similar to those of the monoclonals in that binding is observed to both the 16-19 and 44-47 peptide regions, although peptides 42 and 43 are also recognized by some antisera. There are, however, additional epitopes recognized as well. It cannot be determined if all epitopes are recognized by the polyclonal antisera are linear or conformational. The epitopes cover a significant portion of one face of the PSA molecule.

A series of 75 N-terminally biotinylated 15 mer amides covering the entire sequence of PSA was synthesized, SEQ ID NOS: 19-93. The biotin was attached to the epsilon amino group of an additional alpha-acetyl-lysine on the N-terminus. In an ELISA format the peptides were captured on a streptavidin lawn, followed by the anti-PSA antibodies. Quantitation of peptide-bound antibodies was achieved using HRP-conjugated antisera. A summary of the binding of monoclonal antibodies and polyclonal antisera to PSA epitopes is shown in Figures 2A and 2B. A model of PSA was constructed using homology modeling. The serine proteases tonin and kallikrein were used, with tonin as the template and kallikrein serving as the source of a loop present in PSA but absent in tonin. A loop search was done for PSA (83-88), a sequence for which no serine protease has a homologous region. Two similar structures were generated, differing only in the 83-88 loop. These models are similar to a published PSA model, with the major difference bing the 83-88 loop.

Seven of the nine monoclonals recognized only a single peptide sequence, defined by binding to peptides 16-19 and 44-47. The common amino acid sequences within these peptides are SLFHPE, SEQ ID NO: 2, PSA (55-60), and EEFLTP, SEQ ID NO: 3, PSA (139-144). An examination of the molecular models shows these sequences to be surface exposed. One of the monoclonals, 365, recognizes conformational epitopes. Monoclonal antibody 365 recognizes the sequences TKFMLCAGR (SEQ ID NO: 15), PSA (169-177), and TKVVHYRKW (SEQ ID NO: 16), PSA (220-228) and also the flexible loop LKNRFL (SEQ ID NO: 1), PSA (82-87). Examination of the model shows that only TKF, PSA (160-171), and VHYRKW, SEQ ID NO: 17, PSA (223-228), are surface exposed. Although noncontinuous, these sequences are topologically adjacent, consistent with them being a conformational epitope. The binding of monoclonal antibody 365 to

SEQ ID NO: 1 is significant since the loop region comprising SEQ ID NO: 1 is only conformationally available in free PSA. In addition to the linear epitopes recognized by the monoclonal antibodies, the polyclonal sera tested define additional epitopes in the 1-126 region.

PSA and HK2 share a 79% amino acid identity. Several of the PSA epitopes of the anti-PSA antibodies are highly homologous or identical to corresponding sequences in HK2, suggesting the possibility of cross reactivity to HK2. The fine mapping of anti-PSA antibodies allows for the selection of antibodies to unique PSA sequences, avoiding potential cross reactivity.

The disclosure of each patent, patent application and publication cited or described in this document is hereby incorporated herein by reference, in its entirety.

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CENTOCOR,INC., et al.

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(iii) NUMBER OF SEQUENCES: 94

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(v) COMPUTER READABLE FORM:
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(D) SOFTWARE: WordPerfect 6.0/6.1

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Leu Lys Asn Arg Phe Leu
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Leu Phe His Pro Glu
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Glu Leu Phe Leu Thr Pro
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Pro Trp Gln Val Ala Ile Tyr His Tyr Ser Ser Phe Gln Cys Gly Gly
1 5 10 15

Val

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Pro Trp Gln Val
1

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys Gly Gly Val
1

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys Gly Gly Val
1

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Gly Gly Val
1

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Leu Phe His Pro Glu Asp Thr Gly
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ser Glu Pro Ala Glu Leu
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Glu Phe Leu Thr Pro
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Val His Tyr Arg Lys Trp
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Thr Lys Phe Met Leu Cys Ala Gly Arg
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Thr Lys Val Val His Tyr Arg Lys Trp
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Val His Tyr Arg Lys Trp
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 237 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1 5 10 15

Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His
20 25 30

Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val
35 40 45

Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu Asp Thr Gly Gln
50 55 60

Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asp Met Ser
65 70 75 80

Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser Ser His Asp
85 90 95

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala Val
100 105 110

Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
115 120 125

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro
130 135 140

Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys
145 150 155 160

Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly
165 170 175

Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro
180 185 190

Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu
195 200 205

Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His
210 215 220

Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
225 230 235

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val Leu Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Glu Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

His Ser Gln Pro Trp Gln Val Leu Ala Val Ala Ser Arg Gly Arg Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Pro Trp Gln Val Leu Ala Val Ala Ser Arg Gly Arg Ala Val Cys Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Arg Ala Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gly Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ala His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu Asp Thr Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Arg His Ser Leu Phe His Pro Glu Asp Thr Gly Val Gln Val Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ser Leu Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asp Met Ser Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

His Ser Phe Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser Ser His Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Arg Phe Leu Arg Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Arg Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr Asp Ala Val Lys Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Ser Glu Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Ile Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu His
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gln Cys Val Asp Leu His Val Ile Ser Asn Asp Val Cys Ala Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Asp Leu His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys

1 5 10 15

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Val His Pro Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Thr Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Glu Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val

1 5 10 15

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Val His Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Arg Ala Tyr Ser Glu
1 5

What is claimed is:

1. A monoclonal antibody having the characteristics of monoclonal antibody 365 that binds to a determinant found in free prostate specific antigen wherein said determinant comprises amino acids 82-87 of free prostate specific antigen or a fragment thereof.

2. A hybridoma producing a monoclonal antibody which binds to free prostate specific antigen.

3. A monoclonal antibody produced by the hybridoma of claim 2.

4. A hybridoma producing a monoclonal antibody having the characteristics of monoclonal antibody 10 and that binds to a determinant found in prostate specific antigen wherein said determinant comprises amino acids 139-144 of prostate specific antigen.

5. A monoclonal antibody produced by the hybridoma of claim 4.

6. A hybridoma producing a monoclonal antibody having the characteristics of monoclonal antibody 11 and that binds to a determinant found in prostate specific antigen wherein said determinant comprises amino acids 139-144 of prostate specific antigen or a fragment thereof.

7. A monoclonal antibody produced by the hybridoma of claim 6.

8. A hybridoma producing a monoclonal antibody having the characteristics of monoclonal antibody 15.2 and that binds to a determinant found in prostate specific antigen wherein said determinant comprises amino acids 55-60 of prostate specific antigen or a fragment thereof.

9. A monoclonal antibody produced by the hybridoma of claim 8.

10. A hybridoma producing a monoclonal antibody having the characteristics of monoclonal antibody 16 and that binds to a determinant found in prostate specific antigen wherein said determinant comprises amino acids 139-144 of prostate specific antigen or a fragment thereof.

11. A monoclonal antibody produced by the hybridoma of claim 10.

12. A hybridoma producing a monoclonal antibody having the characteristics of monoclonal antibody 22.2 and that binds to a determinant found in prostate specific antigen wherein said determinant comprises amino acids 139-144 of prostate specific antigen or a fragment thereof.

13. A monoclonal antibody produced by the hybridoma of claim 12.

14. A hybridoma producing a monoclonal antibody having the characteristics of monoclonal antibody 156 and that binds to a determinant found in prostate specific antigen wherein said determinant comprises amino acids 55-60 of prostate specific antigen or a fragment thereof.

15. A monoclonal antibody produced by the hybridoma of claim 14.

16. A hybridoma producing a monoclonal antibody 225 that binds to a determinant found in prostate specific antigen wherein said determinant comprises amino acids 55-60 of prostate specific antigen or a fragment thereof.

17. A monoclonal antibody produced by the hybridoma of claim 16.

18. A monoclonal antibody having the characteristics of a monoclonal antibody selected from the group consisting of monoclonal antibody 10, 11, 16, and 22.2, which monoclonal antibody binds to a determinant found in free and bound prostate specific antigen wherein said determinant comprises amino acids 139-144 of free and bound prostate specific antigen or a fragment thereof.

19. A monoclonal antibody having the characteristics of a monoclonal antibody selected from the group consisting of monoclonal antibody 15.2, 156, and 225, which monoclonal antibody binds to a determinant found in free and bound prostate specific antigen wherein said determinant comprises amino acids 55-60 of free and bound prostate specific antigen or a fragment thereof.

20. A kit for the detection of prostate specific antigen comprising an immunoabsorbant comprising a solid support with a labeled first monoclonal antibody that binds free prostate specific antigen affixed thereto, a solid support with a labeled second monoclonal antibody that binds free and bound prostate specific antigen affixed thereto, which second monoclonal antibody is labeled with a label different from the label of said first monoclonal antibody, and a prostate specific antigen standard.

21. The kit of claim 20 wherein said first monoclonal antibody is a monoclonal antibody that binds amino acids 82-87 of free prostate specific antigen and said second monoclonal antibody is a monoclonal antibody that binds amino acids 55-60 or 139-144 of free and bound prostate specific antigen.

22. The kit of claim 20 wherein said first monoclonal antibody is a monoclonal antibody having the characteristics of monoclonal antibody 365 and said second monoclonal antibody is selected from monoclonal antibody 10, 11, 15.2, 16, 22.2, 156, and 225.

23. A kit for the detection of free prostate specific antigen comprising an immunoabsorbant comprising a solid support with a labeled monoclonal antibody specific for free prostate specific antigen affixed thereto, and a prostate specific antigen standard.

24. The kit of claim 23 wherein said monoclonal antibody binds amino acids 82-87 or a fragment thereof of free prostate specific antigen.

25. The kit of claim 23 wherein said monoclonal antibody is a monoclonal antibody having the characteristics of monoclonal antibody 365.

26. A kit for the detection of free and bound prostate specific antigen comprising a solid support with a labeled monoclonal antibody specific for free and bound prostate specific antigen affixed thereto, and a prostate specific antigen standard.

27. The kit of claim 26 wherein said monoclonal antibody is specific for amino acids 55-60 or 139-144 or fragments thereof of prostate specific antigen.

28. The kit of claim 26 wherein said monoclonal antibody is a monoclonal antibody having characteristics of the monoclonal antibodies selected from monoclonal antibodies 10, 11, 15.2, 16, 22.2, 156, and 225.

29. A method of screening for cancer in a patient comprising contacting a patient sample with a monoclonal antibody specific for amino acids 82-87 or a fragment thereof of free prostate specific antigen under conditions permitting said antibody to specifically bind an antigen in the sample to form a first antibody-antigen complex; contacting said sample with a monoclonal antibody specific for free and bound prostate specific antigen under conditions permitting said antibody to specifically bind an antigen in said sample to form a second antibody-antigen complex; determining the amount of said first antibody-antigen complex in said sample; determining the amount of said second antibody-antigen complex in said sample; and determining the ratio of said first antibody-antigen complex to said second antibody-antigen complex.

30. The method of claim 29 wherein said monoclonal antibody specific for free and bound prostate specific antigen is specific for amino acids 55-60 or 139-144 or fragments thereof of prostate specific antigen.

31. The method of claim 29 wherein said monoclonal antibody is a monoclonal antibody having the characteristics of a monoclonal antibody selected from monoclonal antibody 10, 11, 15.2, 16, 22.2, 156, and 225.

32. A method of claim 29 wherein said cancer is selected from the group consisting of prostate cancer and breast cancer.

33. A method of claim 29 wherein said first monoclonal antibody is labeled with a label selected from the group consisting of biotin and fluorescein.

34. A method of claim 29 wherein said second monoclonal antibody is labeled with a label different from said first monoclonal antibody and said label is selected from the group consisting of biotin and fluorescein.

35. A method for the detection of free prostate specific antigen in a sample comprising incubating a sample with a labeled monoclonal antibody specific for amino acids 82-87 of free prostate specific antigen under conditions and for a period of time for the labeled antibody to bind any free prostate specific antigen in said sample, and quantitatively determining the presence of free prostate specific antigen in said sample.

36. The method of claim 35 wherein said monoclonal antibody is a monoclonal antibody having the characteristics of monoclonal antibody 365.

37. A method for the detection of free and bound prostate specific antigen in a sample comprising incubating a sample with a labeled monoclonal antibody specific for amino acids 55-60 or 139-144 or fragments thereof of prostate specific antigen under conditions and for a period of time for the labeled antibody to bind any free and bound prostate specific antigen in said sample, and quantitatively determining the presence of free and bound prostate specific antigen in said sample.

38. The method of claim 37 wherein said monoclonal antibody is a monoclonal antibody having characteristics of a monoclonal antibody selected from the group consisting of monoclonal antibodies 10, 11, 15.2, 16, 22.2, 156, and 225.

39. A method of making monoclonal antibodies to free prostate specific antigen comprising obtaining the sequence of amino acids 82-87 or a fragment thereof of prostate specific antigen, immunizing an animal with the sequence of amino acids 82-87 or a fragment thereof of prostate specific antigen, screening for the production of an antibody reactive with amino acids 82-87 or a fragment thereof of prostate specific antigen, and cloning an isolated antibody to the amino acids 82-87 or a fragment thereof of prostate specific antigen.

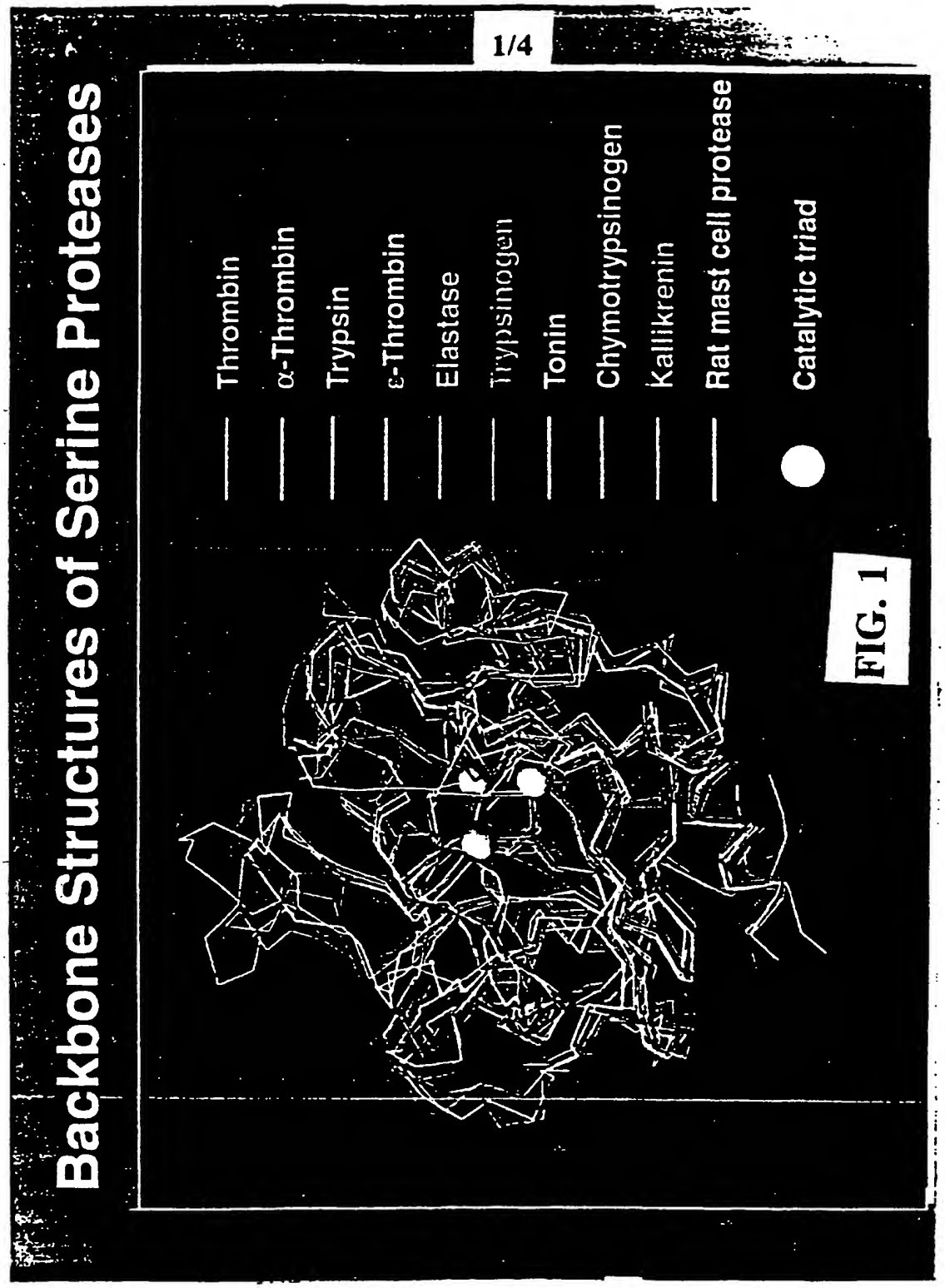

FIG. 1

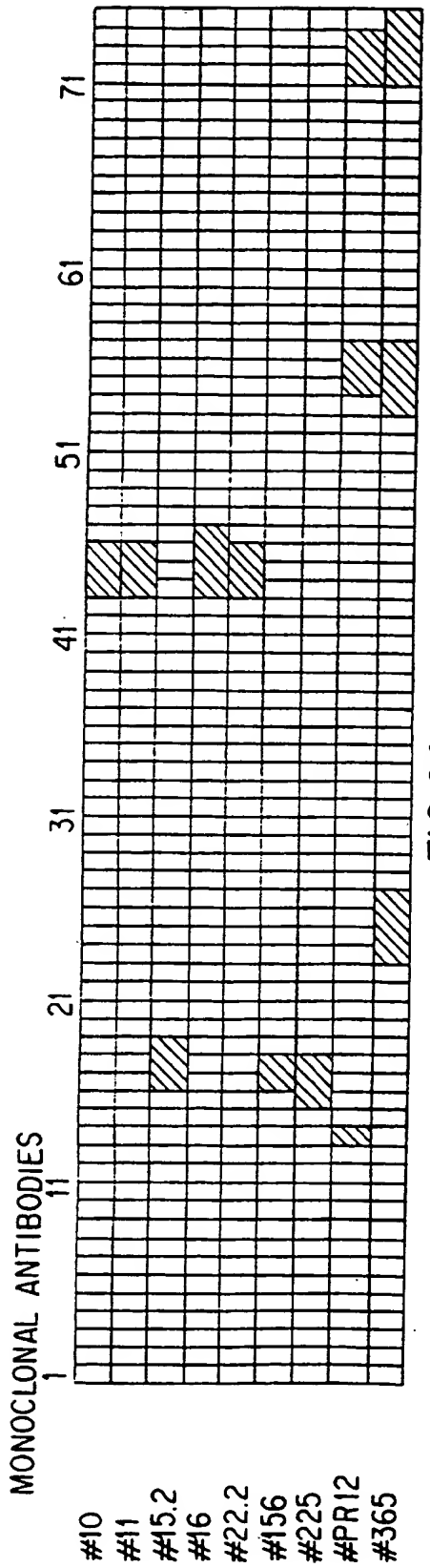

FIG.2A

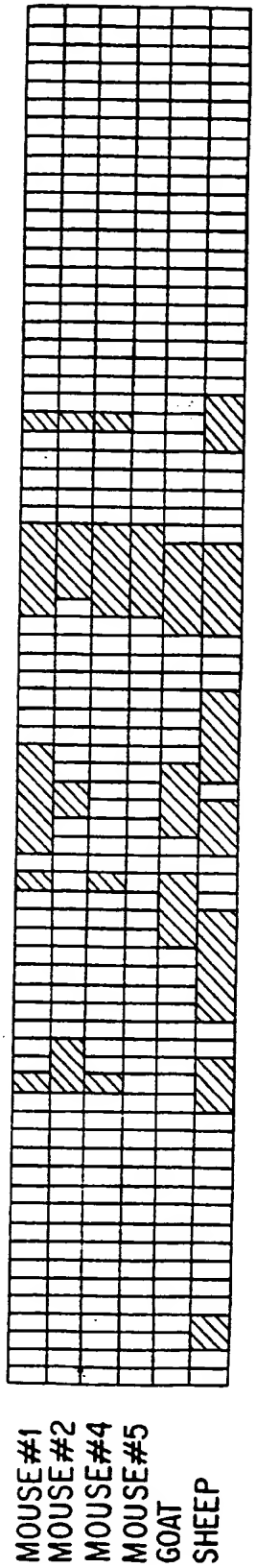

FIG.2B

PSA IVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPE
HK2 ----------A-Y-H-W-H----------KKN-QVW----N--E--

PSA DIGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQ
HK2 ----RVP----------N-----HQS---DE----------KI--V----I.G----

PSA EPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTG
HK2 ----------R-RS----S--LL---M--RAYSE---E-----L---

PSA GKSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP
HK2 --D--G----------P--------K-AV----------A---

FIG.3

Sequence of PSA peptides synthesized. Cys was replaced with L-aminobutyric acid. Surface exposed amino acids are underlined.

Peptide Number	Sequence	Peptide Number	Sequence
1	IVGGWECEKHSQPWQ	39	MDLPTQEPALGTTCY
2	GWECEKHSQPWQVLV	40	PTQEPALGTTCYASG
3	CEKHSQPWQVLVASR	41	EPALGTTCYASGWGS
4	HSQPWQVLVASRGRA	42	LGTTCYASGWGSIEP
5	PWQVLVASRGRAVCG	43	TCYASGWGSIEPEEF
6	VLVASRGRAVCGGVL	44	ASGWGSIEPEEFLTP
7	ASRGRAVCGGVLVHP	45	WGSIEPEEFLTPKKL
8	GRAVCGGVLVHPQWV	46	IEPEEFLTPKKLQCV
9	VCGGVLVHPQWVLTA	47	EEFLTPKKLQCVDLH
10	GVLVHPQWVLTAAHC	48	LTPKKLQCVDLHVIS
11	VHPQWVLTAAHCIRN	49	KKLQCVDLHVISNDV
12	QWVLTAAHCIRNKSV	50	QCVDLHVISNDVCAQ
13	LTAAHCIRNKSVILL	51	DLHVISNDVCAQVHP
14	AHCIRNKSVILLGRH	52	VISNDVCAQVHPQKV
15	IRNKSVILLGRHSLF	53	NDVCAQVHPQKVTKF
16	KSVILLGRHSLFHPE	54	CAQVHPQKVTKFMLC
17	ILLGRHSLFHPEDTG	55	VHPQKVTKFMLCAGR
18	GRHSLFHPEDTGQVF	56	QKVTKFMLCAGRWTG
19	SLFHPEDTGQVFQVS	57	TKFMLCAGRWTGGKS
20	HPEDTGQVFQVSHSF	58	MLCAGRWTGGKSTCS
21	DTGQVFQVSHSFPHP	59	AGRWTGGKSTCSGDS
22	QVFQVSHSFPHPLYD	60	WTGGKSTCSGDSGGP
23	QVSHSFPHPLYDMSL	61	GKSTCSGDSGGPLVC
24	HSFPHPLYDMSLLKN	62	TCSGDSGGPLVCNGV
25	PHPLYDMSLLKNRFL	63	GDSGGPLVCNGVLQG
26	LYDMSLLKNRFLRPG	64	GGPLVCNGVLQGITS
27	MSLLKNRFLRPGDDS	65	LVCNGVLQGITSWGS
28	LKNRFLRPGDDSSHD	66	NGVLQGITSWGSEPC
29	RFLRPGDDSSHDLML	67	LQGITSWGSEPCALP
30	RPGDDSSHDLMLLRL	68	ITSWGSEPCALPERP
31	DDSSHDLMLLRLSEP	69	WGSEPCALPERPSLY
32	SHDLMLLRLSEPAEL	70	EPCALPERPSLYTKV
33	LMLLRLSEPAELTDA	71	ALPERPSLYTKVVHY
34	LRLSEPAELTDAVKV	72	ERPSLYTKVVHYRKW
35	SEPAELTDAVKVMDL	73	SLYTKVVHYRKWIKD
36	AELTDAVKVMDLPTQ	74	TKVVHYRKWIKDTIV
37	TDAVKVMDLPTQEPA	75	VHYRKWIKDTIVANP
38	VKVMDLPTQEPALGT		

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14909

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/574, 33/53; C07K 16/30
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.23; 436/64; 530/387.7, 387.9, 388.26, 388.8, 388.85, 389.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; DIALOG: file biochem; STN; GENESEQ27; SWISS-PROT34
search terms: psa, prostate specific antigen, kallikrein, hK1, hK2, hK3, ACT, antichymotrypsin, antibod?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VILLOUTREIX ET AL. STRUCTURAL INVESTIGATION OF THE ALPHA-1-ANTICHYMOTRYPSIN: PROSTATE-SPECIFIC ANTIGEN COMPLEX BY COMPARATIVE MODEL BUILDING. PROTEIN SCIENCE. MAY 1996. VOL. 5, PAGES 836-851, ESPECIALLY THE ABSTRACT, FIGURE 1, PAGE 840, AND THE LEFT-HAND COLUMN OF PAGE 841.	1-39
Y, P	COREY ET AL. PROSTATE-SPECIFIC ANTIGEN: CHARACTERIZATION OF EPITOPES BY SYNTHETIC PEPTIDE MAPPING AND INHIBITION STUDIES. CLINICAL CHEMISTRY. APRIL 1997, VOL. 43, NO. 4, PAGES 575-584, ESPECIALLY THE ABSTRACT, THE LOWER RIGHT-HAND COLUMN OF PAGE 577, AND PAGE 578.	1-39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 SEPTEMBER 1997	Date of mailing of the international search report 20 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer TONI R. SCHEINER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

BNSDOCID: <WO 9810292A1>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14909

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHRISTENSSON et al. Complex formation between protein C inhibitor and prostate-specific antigen *in vitro* and in human semen. European Journal of Biochemistry. 1994, Vol. 220, pages 45-53, especially the Abstract and Figure 2.	1-39
Y	WO 95/03334 A1 (MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH) 02 February 1995, pages 5 and 6, and Table II.	1-39
Y	WO 92/01936 A1 (H. LILJA et al) 06 February 1992, pages 6 and 7.	1-39

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14909

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.23; 436/64; 530/387.7, 387.9, 388.26, 388.8, 388.85, 389.7